UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
WASHINGTON, D.C. 20460

OFFICE OF CHEMICAL SAFETY AND POLLUTION PREVENTION

MEMORANDUM

DATE: August 12, 2021

SUBJECT: **Aviglycine (AVG):** Report of the Cancer Assessment Review Committee

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On April 21, 2021, the Cancer Assessment Review Committee (CARC) of the Health Effects Division (HED) met to discuss the white paper titled "Aviglycine: Evaluation of carcinogenicity based on weight of evidence". The paper was submitted by the registrant, Valent Biosciences, as a rebuttal to the CARC report on AVG (TXR 0058100, R. Louden, 20-NOV-2020). Attached please find the CARC's response document.

RESPONSE TO VALENT'S REBUTTAL: "AVIGLYCINE: EVALUATION OF CARCINOGENICITY BASED ON WEIGHT OF EVIDENCE"

AVIGLYCINE

PC: 129211

April 21, 2021
Submitted by: Baylor Steele

CANCER **A**SSESSMENT **R**EVIEW **C**OMMITTEE
HEALTH EFFECTS DIVISION
OFFICE OF PESTICIDE PROGRAMS

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EXECUTIVE SUMMARY

On April 21, 2021, the Cancer Assessment Review Committee (CARC) of the Health Effects Division (HED) of the Office of Pesticide Programs (OPP) met to review a rebuttal paper titled "Aviglycine: Evaluation of carcinogenicity based on weight of evidence". The paper requests that the AVG/AVG HCl cancer classification be re-considered and provides key points as to why AVG should not be classified as "Likely to be Carcinogenic to Humans". These key points are as follows:

1. Weaknesses of standard carcinogenicity bioassay in rodents
2. Adequacy of dose levels
3. Method of statistical analysis
4. Human relevance of the tumors observed in carcinogenicity studies
5. Structure-Activity Relationships

After review of the rebuttal paper, the CARC concluded that the information provided by the registrant is insufficient to change the current cancer classification of AVG and AVG HCl. A summary of the AVG rodent cancer studies and the CARC's responses to each of the above points are discussed in the remainder of this document.

I. INTRODUCTION

On April 21, 2021, the Cancer Assessment Review Committee of the Health Effects Division of the Office of Pesticide Programs met to evaluate a rebuttal white paper submitted by Valent BioSciences, LLC (MRID 51465201).

II. BACKGROUND INFORMATION

AVG is currently undergoing registration as a new active ingredient for use as a plant growth regulator in apples. The salt form of AVG, Aviglycine HCl (AVG HCl), is currently registered as a biopesticide, but AVG HCl was not evaluated by the CARC during the pesticide's registration process in 2004 (US EPA 2004). Therefore, the CARC met to evaluate the carcinogenicity of both AVG and AVG HCl on August 20, 2020, which was the first CARC evaluation for AVG and AVG HCl.

The findings from the carcinogenicity studies in CD-1 mice (MRID 47146701) and Sprague Dawley rats (MRID 45698801) were discussed at the August 20, 2020 meeting. In the carcinogenicity study with mice, AVG HCl (89.0% pure) was administered in the diet to groups of 52 mice per sex at dose levels of 0, 0.7, 4.0 or 20.0 mg/kg/day (equivalent to 0.57, 3.3, or 16.3 mg/kg/day AVG) for 78 weeks. The combined chronic carcinogenicity study in rats allocated groups of 65 rats per sex to target AVG HCl (82.1% pure) dose levels of 0, 0.2, 0.7 or 7.0 mg/kg/day (equivalent to 0, 0.16, 0.57, or 5.7 mg/kg bw/day AVG) in diet for 104 weeks. Information on mutagenicity and structure activity relationships was also presented.

The CARC concluded the following:

Mice

- *Liver Tumors*: Male mice had statistically significant trends for hepatocellular adenomas ($p<0.01$) and for hepatocellular adenomas and/or carcinomas combined ($p<0.05$). There were statistically significant pair-wise comparisons of the mid-dose group with the controls for liver adenomas ($p<0.05$) and combined adenomas and/or carcinomas ($p<0.05$). There were also statistically significant pair-wise comparisons of the high-dose group with the controls for liver adenomas ($p<0.01$) and combined adenomas and/or carcinomas ($p<0.05$). In addition, non-neoplastic liver findings at the mid- and high-dose included inflammatory cell infiltration and centrilobular hypertrophy. Adequate historical control data were not available for this study. **The CARC concluded that the liver adenomas and adenomas/carcinomas combined are treatment related in males at the mid-dose (4.0 mg/kg/day AVG HCl; 3.3 mg/kg/day AVG dose equivalent) and high-dose (20 mg/kg/day AVG HCl; 16.3 mg/kg/day AVG dose equivalent).**

- *Adequacy of Dosing*: The doses tested were considered adequate. The initial high dose (16.3 mg/kg/day) was slightly excessive based on mortality and was reduced to 12.2 mg/kg/day starting at week 12, which was considered adequate. Treatment-related microscopic, non-neoplastic findings included hepatic effects (inflammatory cell infiltration and/or centrilobular hypertrophy) and atrial thrombus in mid- and high-dose males, cataracts in high-dose males and females, and retinal atrophy in high-dose females. Lastly, clinical signs of toxicity attributed to exposure were observed in mid- and high-dose males approximately 2 to 3 weeks prior to premature death, including weight loss, piloerection, and/or abnormal respiration. Therefore, dosing was considered adequate based on decreased body weight and the appearance of microscopic lesions in the eye, heart, and liver of both sexes and clinical signs observed in male mice. An adequate number of animals were still available at the end of the study period to evaluate the carcinogenic potential of AVG.

Rats

- *Testicular Tumors*: Male rats had a statistically significant trend ($p<0.01$) for an increase in testicular interstitial cell tumors. There was a statistically significant pair-wise comparison of the high dose group with the controls for testicular interstitial cell tumors ($p<0.05$). Non-neoplastic findings in the testis included testicular tubular atrophy and mineralization. **The CARC concluded that the testicular interstitial cell tumors are treatment related in males at the mid (0.7 mg/kg/day AVG HCl; 0.57 mg/kg/day AVG equivalents) and high dose (7.0 mg/kg/day AVG HCl; 5.7 mg/kg/day AVG equivalents).**

- *Adrenal Tumors*: Female rats had a statistically significant trend ($p < 0.01$) for adrenal gland benign pheochromocytomas and adrenal gland benign and/or malignant pheochromocytomas combined. There was a statistically significant pair-wise comparison of the high dose group with the controls for adrenal gland benign pheochromocytomas and adrenal gland benign and/or malignant pheochromocytomas combined ($p<0.05$). There was also a statistically significant trend for increased adrenal gland malignant pheochromocytomas ($p<0.01$). The historical control information provided did not include study years and therefore were not considered for either the testicular or adrenal tumors. Non-neoplastic findings included increased incidence of focal medullary cell hyperplasia. **The CARC concluded that the adrenal gland benign pheochromocytomas and benign and/or malignant pheochromocytomas combined are treatment related in females at the high dose (7.0 mg/kg/day AVG HCl; 5.7 mg/kg/day AVG equivalents).**

- *Adequacy of Dosing*: The doses tested were considered adequate. Doses were sufficiently high and not excessive for male and female rats as several non-neoplastic microscopic effects and body weight decreases were observed at the high dose (5.7 mg/kg/day). Non-neoplastic findings in males included testicular tubular atrophy, mineralization, increased incidence of seminal vesicle atrophy, increased incidences of bilateral oligospermia, and spermatogenic cell sloughing in the epididymis. Females showed an increased incidence of focal medullary cell hyperplasia in the adrenal gland. Additionally, increased incidences of ocular cataracts were observed in both males and females at the high dose. In the heart, the incidence of ectopic calcification was significantly increased in females at the high dose, though not in males, while both sexes showed a significantly lower incidence of progressive cardiomyopathy.

Mutagenicity: There is no concern for mutagenicity.

Structure Activity: Structure activity relationship data was not submitted for the registration of AVG HCl. Using ChemIDPlus, a search was performed for chemicals with ≥60% structural similarity to AVG. Besides AVG HCl, two similarly structured chemicals were identified, rhizobitoxine (79% similar) and 2-amino-4-methoxy-3-butenoic acid (76% similar). Neither of these chemicals are pesticides/biocides. No mammalian toxicity data are available for rhizobitoxine or 2-amino-4-methoxy-3-butenoic acid.

Classification and Quantification of Carcinogenic Potential: In accordance with EPA's *Final Guidelines for Carcinogen Risk Assessment* (March 2005), the CARC classified AVG and AVG HCl as "*Likely to be Carcinogenic to Humans*". This was based on treatment-related hepatocellular tumors in male mice (adenomas and combined adenomas/carcinomas), testicular interstitial cell tumors in male rats (adenomas), and adrenal pheochromocytomas in female rats (benign, malignant, and benign/malignant combined).

III. THE REGISTRANT'S PROPOSED EVIDENCE SUPPORTING REBUTTAL

In response to the AVG and AVG HCl CARC report, Valent BioSciences submitted a white paper titled "Aviglycine: Evaluation of carcinogenicity based on weight of evidence". The white paper requested that the AVG and AVG HCl cancer classification be re-considered and provided key arguments as to why the active ingredients should not be classified as likely to be carcinogenic to humans. The registrant's key points are listed below followed by CARC's responses.

1. Weakness of standard carcinogenicity bioassay in rodents
2. Adequacy of dose levels
3. Method of statistical analysis
4. Human relevance of the tumors observed in carcinogenicity studies
5. Structure-Activity Relationships

1. <u>Weaknesses of Standard Carcinogenicity Bioassay in Rodents.</u>

In their rebuttal paper, Valent BioSciences stated that rodent carcinogenicity assays are time-consuming, expensive, and subject to ethical (use hundreds of animals) and scientific debate (the limited translatability of rodent assays to man and a rather low reproducibility of 60-70%). The rebuttal paper cited literature indicating that approximately 50% of chemicals that have been tested for carcinogenicity were deemed to be rodent carcinogens (Ames and Gold, 2000; Goodman, 2018). Valent also cited a study whereby a re-evaluation of National Toxicology Program bioassay revealed that if the dose group size were increased from 50 to 200 rodents per group, the number of cancer bioassays deemed to be positive would increase from approximately 50% to close to 100% (Gaylor, 2005).

CARC's Response:
The Agency requires carcinogenicity studies in two rodent species for all food use pesticides and when long-term exposure is anticipated. These studies are designed to determine whether a pesticide has carcinogenic activity when administered over the lifetime of the animal. These rodent bioassays are also used by national and international regulatory agencies to assess

carcinogenicity. These studies remain the "gold standard" for carcinogenicity testing and CARC relies on the findings from these studies as part of its overall weight of evidence when determining the cancer classification of pesticides.

2. Adequacy of Dose Levels

A. Mouse Study

Valent contends that the highest AVG HCl dose in the mouse carcinogenicity study was inadequate due to excessive mortalities, reduction in body weight gain, and reduction in absolute body weight. In the AVG and AVG HCl cancer classification rebuttal paper, excessive mortalities are identified in male mice at the high- and mid-dose, and excessive decreases in body weight gain and absolute body weight are identified at the high dose for male and female mice.

CARC's Response:
In the mouse study, survival never fell below 60% in each of the AVG HCl treatments. This survival rate is above the harmonized guideline (OCSPP 870.4200) recommendation of 50% and 25% survival in mice at 15 and 18 months, respectively to be considered an acceptable study. No dose response trend was observed for mortality in male mice as mortalities in the high-dose exceeded those in the mid-dose. Additionally, the Peto analysis performed on the tumor data in both mouse and rat studies accounts for mortalities when identifying statistically significant relationships between AVG HCl treatments and tumor incidences.

Absolute body weight loss in female mice (19%) at the high dose exceeded that of male mice, but the CARC did not consider this deficit an indication of an excessive dose. Furthermore, body weight loss in mice at the high dose did not reach 10% until week 58 or later, indicating that these are chemical effects that are occuring gradually over time rather than effects associated with excessive toxicity. Finally, no significant effects on weight were observed at the mid-dose, yet increased instances of liver tumors were observed at this dose. Based on the information provided above, there is not sufficient evidence to conclude that any of the doses are excessive.

B. Rat Study

Valent contends that the AVG HCl doses tested in the rat carcinogenicity assays were inadequate due to excessive mortalities, reduction in body weight gain, and reduction in absolute body weight. In the AVG and AVG HCl cancer classification rebuttal paper, excessive mortalities are identified in male rats at the high- and mid-dose, and excessive decreases in body weight gain and absolute body weight are identified at the high-dose for male and female rats.

CARC's Response:
As with the mouse cancer study, survival in the rat cancer study remained above the OCSPP guideline recommendations (50% at 18 months or 25% at 24 months), and effects of mortality on statistically significant relationships between AVG HCl treatments and tumor incidences were accounted for by the statistical analysis (Peto analysis) employed. Furthermore, mortalities in control (55%), low-(71%), and mid-dose (58%) female rats exceeded those in females treated at

the high dose (51%). This information is counter to the point that the high dose is excessive because mortalities increase as treatment level decreases.

The decreases in absolute body weight in both male (37.7%) and female (35.3%) rats could be indicators of dose excessiveness, but weight loss did not reach ~35% until the end of the study, at week 104. Clinical signs including, higher incidences of hunched posture, unkempt coat, rolling gait, and piloerection, were observed during the study, indicating possible dose excessiveness. However, the majority of male rats (81.6%) exhibiting these clinical signs did not do so until late into the study, between days 365 and 546. The mean time to appearance of hunched posture, unkempt coat, rolling gate, or piloerection was 418 days. Furthermore, treatment related instances of testicular tumors were also observed at the mid-dose, yet no significant weight loss occurred at this dose.

3. <u>Method of Statistical Analysis</u>

A. Application of the "Haseman Rule"

Valent contends that the method of statistical analysis used to identify significant instances of tumors in AVG HCl dosed rats and mice is inadequate because the analysis does not account for multiple tissue comparisons, which will result in an unacceptably high experimental false positive rate. In their rebuttal, Valent proposed application of the Haseman Rule (Haseman, 1983, 1990), a statistical approach whereby levels for statistical significance are set at $p<0.01$ (for pairwise comparisons) and $p<0.005$ (for trend tests) when background (control, historical control) rate tumor incidences exceed 1%.

CARC's Response:
As standard practice, the CARC recognizes levels of statistical significance at $p< 0.05$ for both trend and pairwise tests. However, the p-values observed for the AVG cancer studies fall far below 0.05. Even when the Hasemen Rule is applied, statistically significant (trend and pairwise) hepatocellular tumors are observed in male mice at the high-dose. Valent states that this dose should not be included in the cancer analysis because increased incidences of hepatocellular adenomas at the high dose is irrelevant due to high mortalities and weight loss. As discussed in more detail in Section 2 above, the CARC does not consider the high dose to be excessive.

B. Pairwise Tests for Statistical Analysis

Valent also contends that only pairwise tests should be used in the analysis because the significant trends observed at the high- and mid-dose in male mice were due to excessive toxicity that exceeded the MTD (producing reduced survival and reduced body weight). Valent cites US FDA guidance (2001) whereby pairwise tests may be more appropriate.

CARC's Response:
As mentioned above, the CARC does not consider the doses utilized to be excessive. Consistent with EPA's 2005 Guidelines for Carcinogenic Risk Assessment, the CARC relies on both trend and pairwise tests when analyzing tumor data. In the cancer assessment of AVG, the CARC applied statistical approaches (e.g., Peto analysis, $p<0.05$) that account for survival disparities

and take both type I (i.e., false positive) and type II errors into consideration. Therefore, the CARC considers the method of statistical analysis to be appropriate, and no additional analyses are warranted.

4. <u>Human Relevance of the Tumors Observed in Carcinogenicity Study with AVG HCl</u>

A. Hepatocellular tumors in male mice

1. *High background rate of tumor incidences*

Valent Biosciences suggests that tumors in male mice at the mid- and high- AVG HCl doses are associated with a high background tumor rate as observed in historical control animals. Tumors in historical control animals are >1% spontaneous incidence, and therefore these tumors should be considered common. Factors operating against this high background rate can cause an elevation in tumor incidences in test chemical treated animals.

CARC's Response:
The CARC considers the concurrent controls to be the primary comparison group when determining if tumors are treatment related. The CARC also considers relevant historical control information which can inform whether the tumor incidences observed in the concurrent control group are within the expected range for a specific rodent strain at the time the study was conducted. The mice in the control group in the AVG HCl cancer study had tumor incidences that were within the expected range of incidences based on the historical control animal data provided by Valent.

2. *Relevance of Liver Tumor Mode of Action*

Valent further states that non-genotoxic liver carcinogens are known to operate by MOAs that have little relevance to human exposures. While the exact MOA for liver tumor production by AVG HCl is unknown, the rebuttal paper states that mutagenic-, estrogenic-, immune suppressive-, and cytotoxic-MOAs are not likely attributed to AVG HCl based on the existing data. The paper states that AVG HCl reporter gene assays were negative for effects on estrogen and androgen receptors, there was no evidence of an effect on immune tissues in the short- and long-term bioassays, and AVG HCl is nongenotoxic. Valent suggests that the liver tumors are CAR- or PPARα-mediated based on liver effects observed in 28-day and 90-day AVG toxicity studies with mice. Lastly, Valent proposes that AVG HCl be classified based on a dose threshold that will likely not cause cancer. Since AVG HCl had no effects on the liver, including weights and histopathology, at a dose level of 0.7 mg/kg/day, AVG HCl is not likely to be carcinogenic below 0.7 mg/kg/day.

CARC's Response:
As described in EPA's 2005 Guidelines for Carcinogenic Risk Assessment, chemical specific data are needed to support a proposed non-genotoxic tumor mode of action for tumors. The mode of action framework is used by the CARC to determine the human relevance of tumor responses and the appropriate methodology for cancer risk assessment (i.e., linear vs. non-linear approaches). In the case of AVG HCl, no chemical specific tumor mechanistic data were

submitted to support a propose non-genotoxic mode of action for liver tumors in rodents.

B. Other tumor types

1. *Tumor occurrences linked to endocrine changes from weight loss*

The rebuttal paper indicates that body weight changes can alter physiology of the rat producing stress and effects on the endocrine status of the animals. It is proposed that these endocrine changes could be linked to the tumor occurrences. Several studies show that life-long restriction in caloric intake improves 2-year survival, controls adult body weight and delays the onset of diet- and age-related spontaneous diseases and tumors (Molon-Noblot et al., 2003). The paper states that at the highest AVG HCl dose, both male and female rats had significantly decreased numbers of animals with tumors. Decreases were evident for mammary fibroadenoma in females (10/63 vs. 36/65 in controls; $p<0.001$); thyroid C-cell adenoma in males (3/63 vs. 10/61 in controls; $p<0.05$); and anterior pituitary adenoma in both males (7/65 vs. 12/64 controls; n.s.) and females (21/65 vs. 39/65 controls; $p<0.001$). Valent considers dosing at the high dose to be excessive based on the decrease in body weight gain. Based on this information, the rebuttal paper suggests that body weight decreases were likely related to the decreased numbers of spontaneous tumors in the high-dose animals because the high AVG HCl dose induced an outcome that is similar to life-long dietary restriction.

Valent cites studies from the literature indicating that luteinizing hormone (LH) is a main causative factor for production of rat testicular interstitial tumors (Leydig cell tumors) (Cook et al., 1999). Furthermore, food restriction resulting in decreases in body weight can enhance LH secretion (McShane and Wise, 1996) and reduce pituitary tumors (Molon-Noblot et al., 2003). The rebuttal paper suggests that LH imbalances occurred in AVG HCl dosed rats because a reduction in the incidence of pituitary tumors were observed in males and females treated at the high dose. The paper also cites studies to demonstrate the association of reduced food restriction with reduced incidences of pituitary tumors (Molon-Noblot et al., 2003), development of ovarian neoplasms (Rehm et al., 1984), and delays in reproductive senescence (McShane and Wise, 1996).

Valent concludes that the rat reproduction study is not informative for carcinogenicity evaluation of AVG HCl at 7 mg/kg/day AVG HCl because findings (testicular degeneration with atrophy of the seminal vesicle and decreased prostate weights) at the higher dose (8 mg/kg/day AVG HCl) in the rat reproduction study are possibly linked to markedly decreased body weights, direct test article toxicity to these organs, or hormonal factors associated with a treatment effect occurring elsewhere on the hypothalamus-pituitary-testis-axis. Luteinizing hormone imbalances are unlikely for AVG HCl, as evidenced by no possible anti-androgenic effects such as no direct cytotoxic effects on testis and no reproductive abnormality in rats. Furthermore, Valent states that AVG HCl does not interact with the androgen receptor when tested *in vitro* assays. Lastly, Valent claims that 8 mg/kg/day AVG HCl exceed the MTD based results from on the 90-day study with AVG HCl.

CARC's Response:
In order to support claims that the tumor incidences observed in rats are mediated by LH imbalances associated with weight loss, chemical specific data in rats are needed to evaluate hypothesized modes of action for each tumor type as described in the EPA's 2005 Guidelines for Carcinogenic Risk Assessment. The CARC recommends the data be presented in a manner consistent with the International Programme on Chemical Safety (ICPS) framework for analyzing the relevance of cancer mode of action for humans.

2. *Testicular interstitial cell tumors in male rats*

Valent suggests that interstitial cell tumors (ICTs) observed in the AVG HCl rat carcinogenicity study are not relevant to human health cancer assessment because human epidemiology studies and rodent experiments have demonstrated that various chemicals, including several pharmaceuticals, induce ICTs in rats but do not induce IC hyperplasia or IC adenoma in humans (Andersson et al., 2017; Cook et al., 1999). The rebuttal paper indicates that these differences are likely due to differences in physiology between rats and humans as rats appear to have more than 20000 LH receptors/IC, while humans have closer to 2000 LH receptors/IC (Huhtaniemi et al., 1982), which should render the rat cells more susceptible to changes in circulating LH. Human chorionic gonadotropin, which binds to the LH receptor, is mitogenic in rats (Christensen and Peacock, 1980) but not so in humans (Heller and Leach, 1971). Valent cites data indicating that the spontaneous back-ground rate of ICTs in is 5 to >80% in Sprague-Dawley or Fisher rats (Clegg et al., 1997; Cook et al., 1999; Maronpot et al., 2016; Nolte et al., 2011), respectively, while the rate in humans is approximately 4 in 10000000 (Cook et al., 1999).

The rebuttal paper provided further discussion that refers to data from the 90-day rat study whereby no abnormalities in the testes up to the high dose (9.2 mg/kg/day AVG HCl) were observed, demonstrating no tumorigenic precursor findings after 90-day treatment at this dose. Valent states that even if tumors are considered treatment related, the high- and mid-dose at which tumors were observed is considerably higher than what would be expected for human exposure (0.000128 mg/kg/day). Valent proposes that AVG HCl is not likely to be carcinogenic below 0.7 mg/kg/day because AVG HCl had no effects on the testis at this level and there is no precursor key event of tumor production at this dose.

3. *Adrenal gland pheochromocytoma in female rats*

Valent suggests pheochromocytoma in female rats are occuring through mechanisms other than those that are precursors to the carcinogenic process. The rebuttal paper discussed a study (Greim et al. 2009) whereby pheochromocytomas occur with relatively higher frequency in male rats, especially when the following conditions are involved: hypoxia, uncoupling of oxidative phosphorylation, disturbance in calcium homeostasis, and disturbance of the hypothalamic endocrine axis. Valent states that these underlying biochemical mechanisms indicate other substances that interfere with biochemical endpoints, including enzymes involved in catecholamine synthesis, receptor tyrosine kinase, hypoxia inducible factor, succinate dehydrogenase, fumarate hydratase, and pyruvate dehydrogenase, produce pheochromocytomas. Valent cites a study indicating that pheochromocytomas that occur in animal experiments currently appear to have little relevance for conditions at the workplace or for human exposures in general (Greim et al., 2009). The rebuttal paper suggested that compounds that act secondary to exaggerated pharmacologic effects, altered nutritional status, or physiologic disturbances would not present a risk for humans. All of the above mechanisms discussed would exhibit a threshold (McClain, 1994). Valent states that the MOA for pheochromocytoma-induction in female rats treated with AVG HCl is not known, but the pheochromocytomas were observed only at high dose levels which exceeded the MTD, and thus cannot be distinguished from non-specific effects of severe stress. The dose at which pheochromocytomas were observed is considerably higher than what would be expected for human exposure (0.000128 mg/kg/day). Valent proposes that AVG HCl is not likely to be carcinogenic below 0.7 mg/kg/day because there is no precursor key event of tumor production, based on the US EPA risk assessment methodology.

CARC's Response:
Chemical specific data in rats are needed to evaluate hypothesized modes of action discussed above as described in the EPA's 2005 Guidelines for Carcinogenic Risk Assessment. The CARC recommends the data be presented in a manner consistent with the International Programme on Chemical Safety (ICPS) framework for analyzing the relevance of cancer mode of action for humans.

4. *Historical control animals and method of statistical analysis*

The rebuttal paper stated that there is wide variation in incidence of spontaneous pheochromocytomas in the historical control data and that the incidences of tumors observed at the high AVG HCl dose were within the historical control range. When the Haseman Rule is applied, the finding was not statistically significant (>0.01), suggesting little difference from spontaneous variation.

CARC's Response:
CARC considers the concurrent controls to be the primary comparison group when determining if tumors are treatment related. The CARC also considers relevant historical control information which can inform whether the tumor incidences observed in the concurrent control group are within the expected range for a specific rodent strain at the time the study was conducted. CARC recognizes P<0.05 to be the level of statistical significance for both trend and pairwise analyses.

5. <u>Structure Activity Relationship</u>

In order to predict structure activity relationship, Valent used two *in silico* approaches, a rule-based system and a statistics-based system:

1. Derek Nexus: 6.1.0, a rule-based system for prediction of toxicology and genotoxicity implemented in Nexus (v.2.3.0); Selected Knowledge Base(s) Derek KB 2020 1.0

2. CASE Ultra: 1.8.0.0, a statistics-based system for prediction of toxicology; Model Version: 1.7.0.5.150.400

Derek Nexus, rule-based analysis, did not give any alerts for the following endpoints:

- 5alpha-Reductase inhibition
- glucocorticoid receptor agonism
- adrenal gland toxicity
- hepatotoxicity
- androgen receptor modulation
- peroxisome proliferation
- carcinogenicity
- photocarcinogenicity
- developmental toxicity
- testicular toxicity

The analysis results using the CASE Ultra, statistics-based system, are summarized in Table A. AVG HCl was not in the domain of male mice, male and female rat carcinogenicity. CASE Ultra predictions for female mice carcinogenicity are inconclusive, because a structure "C2H-O" alerts positive 5 out of 7 molecules. However, since 3 out of these 5 positive chemicals are supported by other positive alerts of which none is present in AVG HCl and the existing experimental data demonstrated that AVG HCl was non-carcinogenic for female mice, it is considered that these positive results can be discounted.

Table A. Summary of the results in CASE Ultra system prediction

Endpoints	Results
Male rat carcinogenicity	Out of Domain
Female rat carcinogenicity	Out of Domain
Male mouse carcinogenicity	Out of Domain
Female mouse carcinogenicity	Inconclusive
Rat male fertility	Out of Domain
Rat female fertility	Negative
Human acute liver damage	Out of Domain
Estrogen receptor α agonist	Known Positive
Estrogen receptor α antagonist	Negative
Estrogen receptor β agonist	Out of Domain
Estrogen receptor β antagonist	Out of Domain
Estrogen agonist	Inconclusive
Estrogen antagonist	Known Negative
Estrogen binding	Negative
Androgen receptor agonist (Human cell)	Out of Domain
Androgen receptor agonist (rat cell)	Inconclusive
Androgen receptor antagonist (Human cell)	Out of Domain
Androgen receptor antagonist (rat cell)	Out of Domain
Aryl hydrocarbon	Out of Domain

The rebuttal paper also summarized data from TOX21 receptor binding assays, which indicate that AVG is negative for androgen receptor activity but is positive for estrogen receptor alpha (ERα) activity (agonist) at 20 µM and above. Assays conducted by Valent were negative for effects on estrogen and androgen receptors at a concentration of 10 µM and below. Given that ERα is the predominant isoform (Wang et al., 1999) in the uterus and there were no uterus adverse effects observed in the short- and long-term toxicology studies, Valent states that AVG

HCl is not considered to have estrogen agonistic activity and estrogen agonistic effects are not involved in inducing tumor formation. Valent suggests that the combined results from structure activity relationship predictions and from *in vitro* screening assays indicate that there are no concerns for AVG carcinogenicity.

CARC's Response:
The limited *in silico* information above did not provide convincing evidence that the statistically significant tumors observed in the rodent carcinogenicity studies are not treatment related.

V. CONCLUSION

The information provided in the white paper does not alter CARC's previous conclusions regarding the treatment-related tumors identified in the rodent cancer studies. The cancer classification for Aviglycine/Aviglycine HCl remains "Likely to be Carcinogenic to Humans".

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